Challenges in Identifying Food Components Responsible for Health Effects in Whole Foods

Elizabeth Jeffery, Bioactive Food Components March 24-25, 2005

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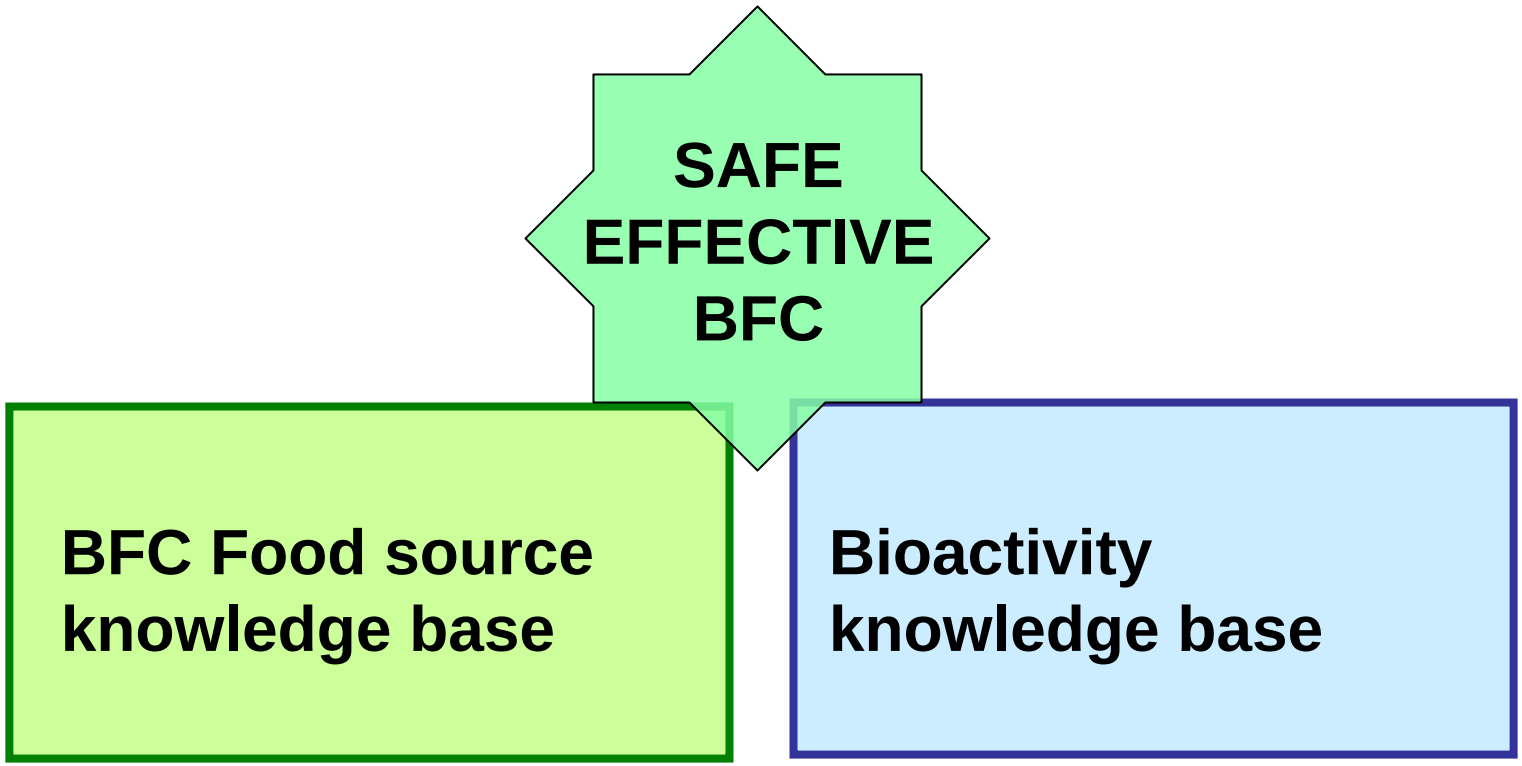
Our single greatest need in the field of BFC study is information: we need a solid scientific basis of knowledge about the food and the individuals who will consume the food



Q Are Cranberries an effective functional food?

Is protection from urinary infection an old wives tale or alternative medicine ?

Heath Family: *Ericaceae*

- ***cranberries, blueberries, huckleberries, bilberries***

A

Gold standard response: Clinical trials can effectively persuade us of efficacy; history of use assumes safety.

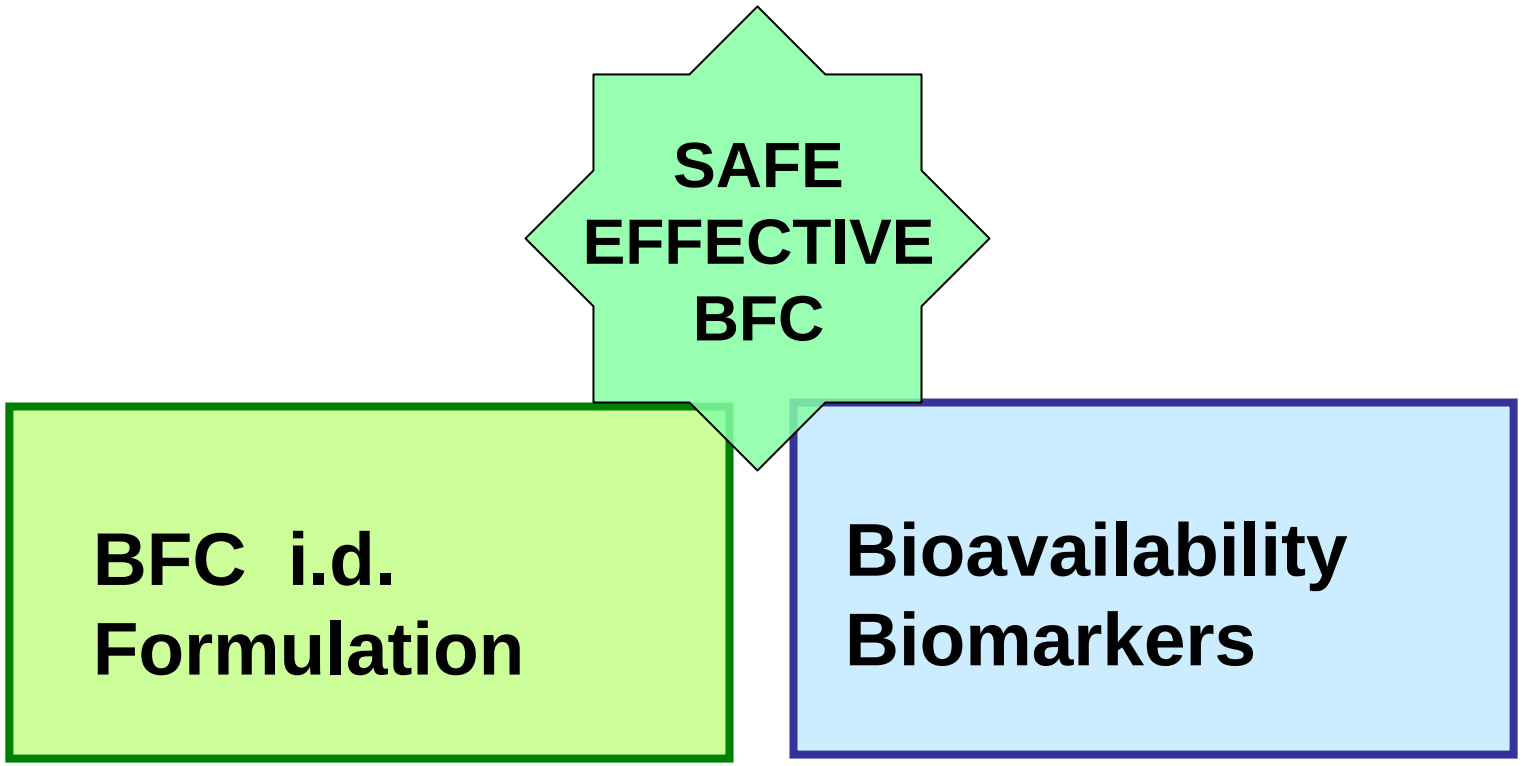
Clinical Trials of Cranberries in Prevention of Urinary Tract Infection

Reference	Patient Group	*n*	Trial Design	Cranberry Juice Dose	Outcome
Dignam *et.al*. (1977)[27]	Elderly men and women	538	Historical Comparisons	6 capsules or 220 ml/day	Fewer UTIs (P=0.001)
Avon *et.al.* (1994)[25]	Elderly women	153	Placebo-controlled	300ml/day	Reduced bacteriuria (P=0.004)
Haverkorn and Mandigers (1994)[30]	Elderly men and women	7	Crossover (not blinded)	15 ml twice daily	Fewer UTIs (P=0.004)
Walker *et.al*. (1997)[28]	Middle-aged women	10	Crossover (double-blind)	400 mg capsules/day	Fewer UTIs (P<0.005)

Fruits, Vegetables & Cancer Prevention: Epidemiology is undecided

Variety or Category	% Positive
Vegetables	80% (59/74)
Fruits	64% (36/56)
Raw vegetables	87% (40/46)
Cruciferous Vegetables	69% (38/55)
Allium Vegetables	77% (27/35)
Green vegetables	77% (68/88)
Carrots	81% (59/73)
Tomatoes	71% (36/51)
Citrus Fruit	66% (27/41)

The 1997 World Cancer Research Fund and the American Institute for Cancer Research (WCRF/AICR) report: Food, Nutrition and the Prevention of Cancer: a global perspective, p442. by John D Potter and other panel members


SAFE
EFFECTIVE
BFC
BFC i.d.
Formulation
Bioavailability
Biomarkers

BFC i.d.
Formulation

What is/are the active component(s), the food ?
(garlic; olive oil; full Latin name if botanical)

Do multiple components have different roles, affecting different endpoints ?

Do components (active and/or inactive) interact ?
(matrix effects; synergism between components)

Some foods contain multiple, differently acting, bioactive components

Tea contains not only polyphenolic anticarcinogens such as Epigallocatechin-3-gallate, but 2 – 10 mM L-theamine also.

Alkylamines have been found to boost the immune system.

Kamath et al, 2003. PNAS 100:6009-6014

WHOLE FOOD

+ − 0 0 + +

ANTAGONISM 0

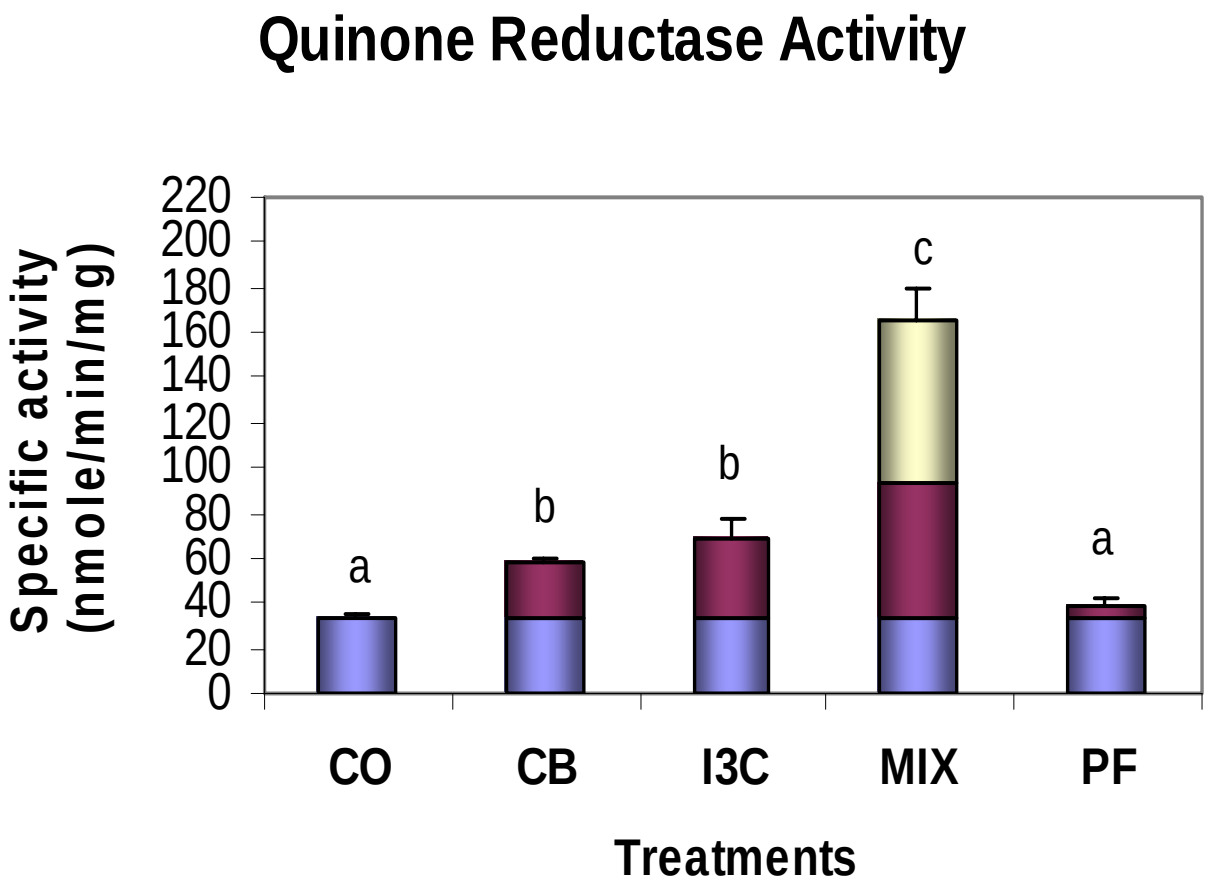
+++ SYNERGY

+++++ ENHANCEMENT

\+ INHIBITION/ MASKING

ACTIVITY (FOOD) =?= ACTIVITIES (Σ KNOWN BFCs)

Synergism in bioactivity



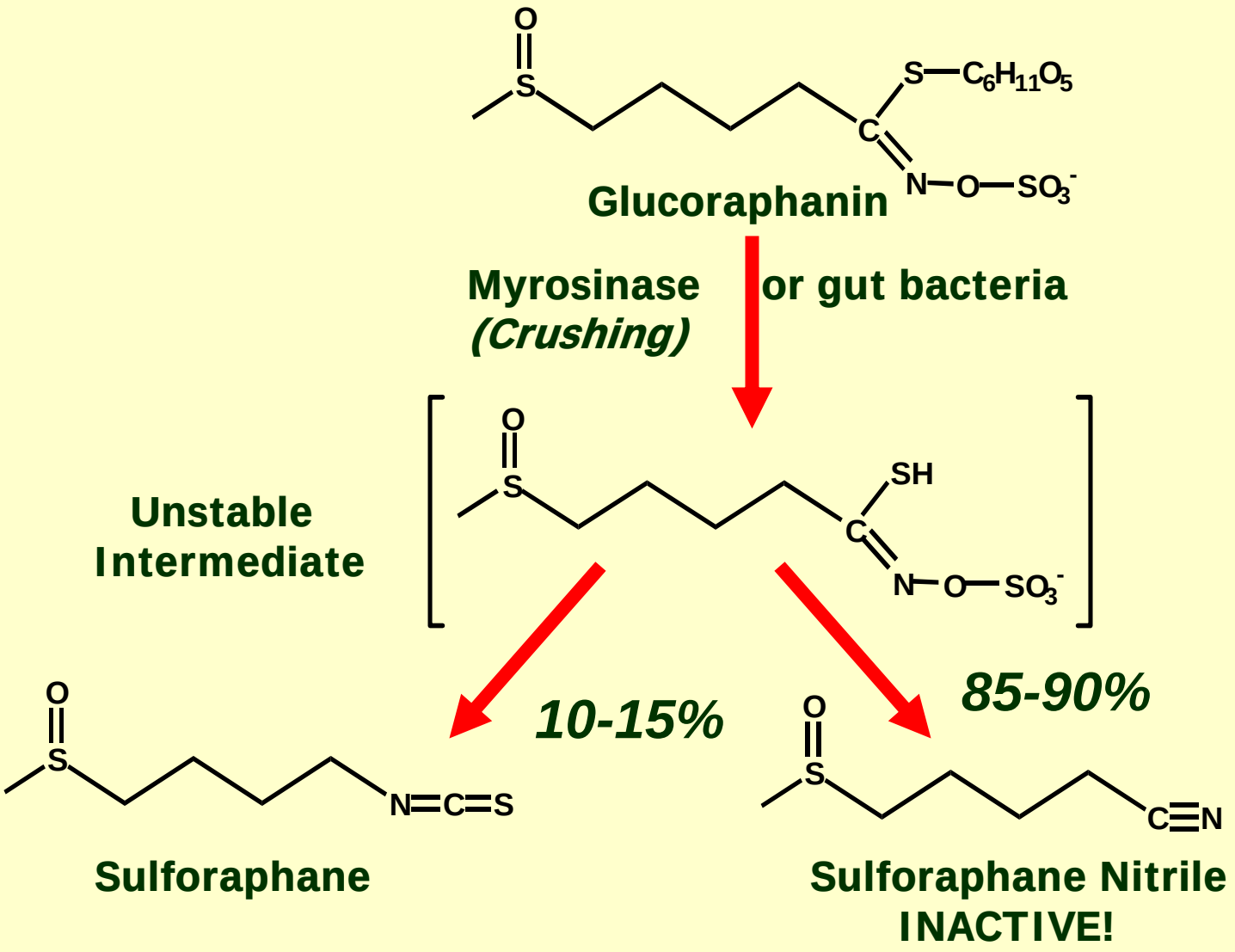
Mean ± SE, n=4 male F344 rats; ($p \leq 0.05$, ANOVA & LSD)
Dose: 50 mg/kg rat crambene; 56 mg/kg rat Indole-3-carbinol

Nho and Jeffery, 2001

Bioavailability Biomarkers

Biomarkers of exposure / bioavailability
(dietary intake measures;
systemic levels; pharmacokinetic studies)
Biomarkers of efficacy
(endpoint choices; validation)
Biomarkers of unexpected / unwanted effects
(the Vioxx factor; safety profile)
Biomarkers of Risk
(genotyping, environment)

Glucoraphanin Hydrolysis



Matusheski et al, 2001

Bioavailability Biomarkers

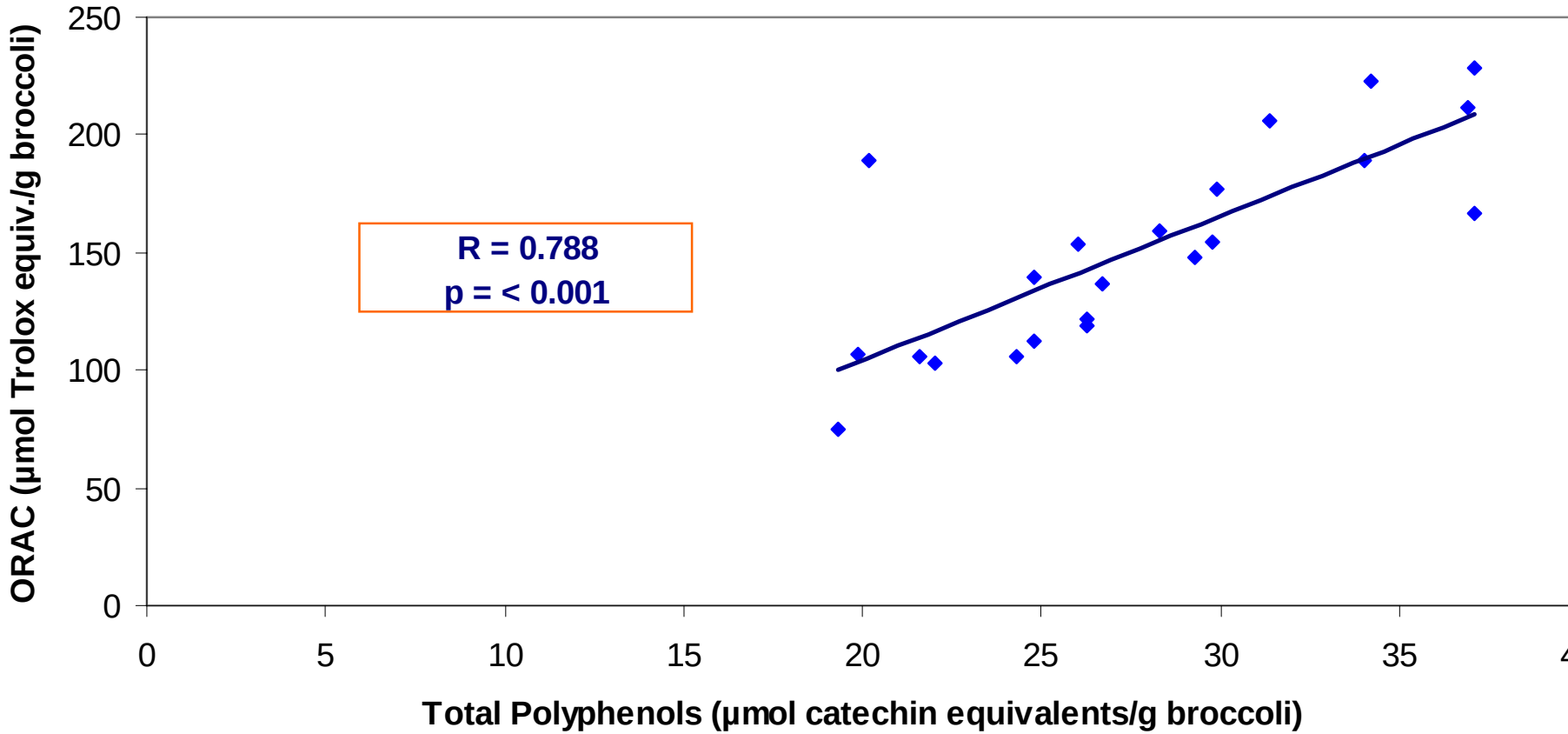
Biomarkers of exposure
(dietary intake measures)
Bioavailability:
systemic levels and pharmacokinetics
(lycopene, sulforaphane)
Biomarkers of efficacy
(endpoint choices; validation)
Biomarkers of unexpected / unwanted effects
(the Vioxx factor; safety profile)
Biomarkers of Risk
(genotyping, environment)

“Antioxidants per serving”

What does this mean ?

Dark Chocolate	951 mg/40 g
Milk Chocolate	394 mg/40 g
Hot Chocolate	45 mg/240 mL
Black Tea	943 mg/240 mL
Red Wine	431 mg/240 mL

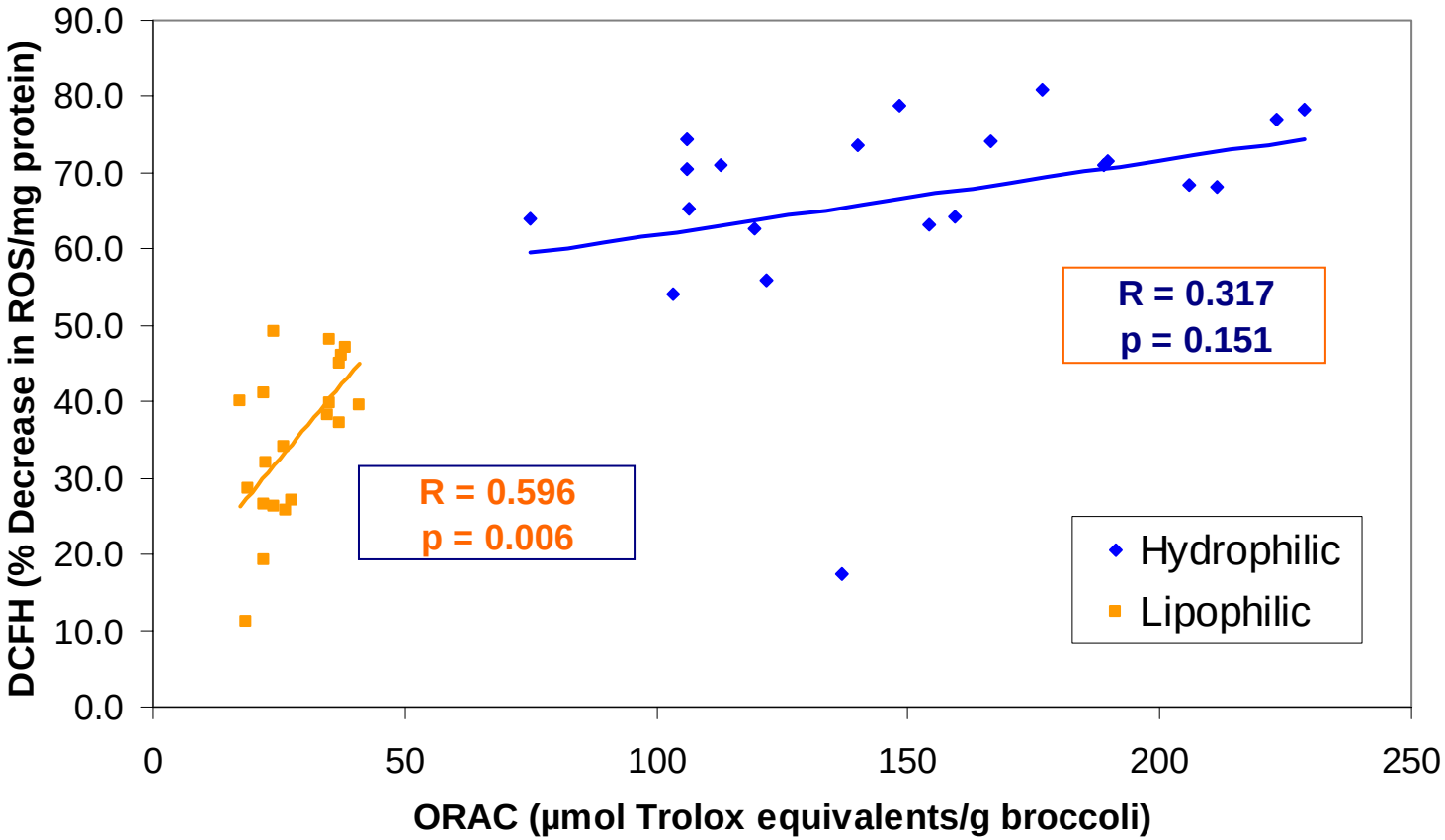
The Oxygen Radical Absorbance Capacity (ORAC) correlates with estimate of total polyphenols



Not validated for any bioassay of antioxidant activity

Eberhardt et al, 2005 Experimental Biology

ORAC showed poor correlation with a cellular measure of antioxidant activity



22 broccoli genotypes; extracts analyzed by ORAC and by a dichlorofluorescein measure of ROS quenching by extracts in HepG2 cells.

Bioavailability Biomarkers

Biomarkers of exposure
(dietary intake measures)
Bioavailability:
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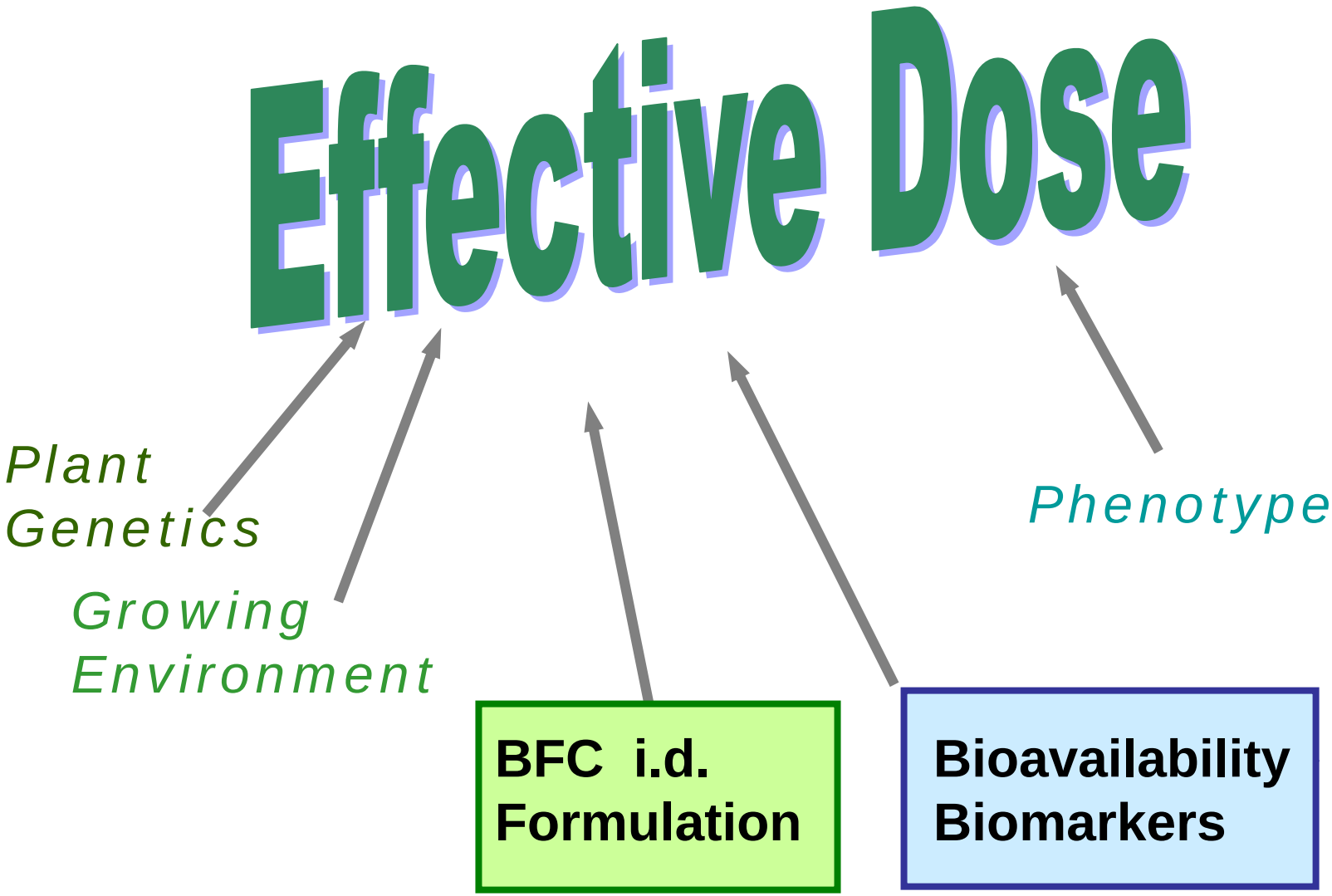
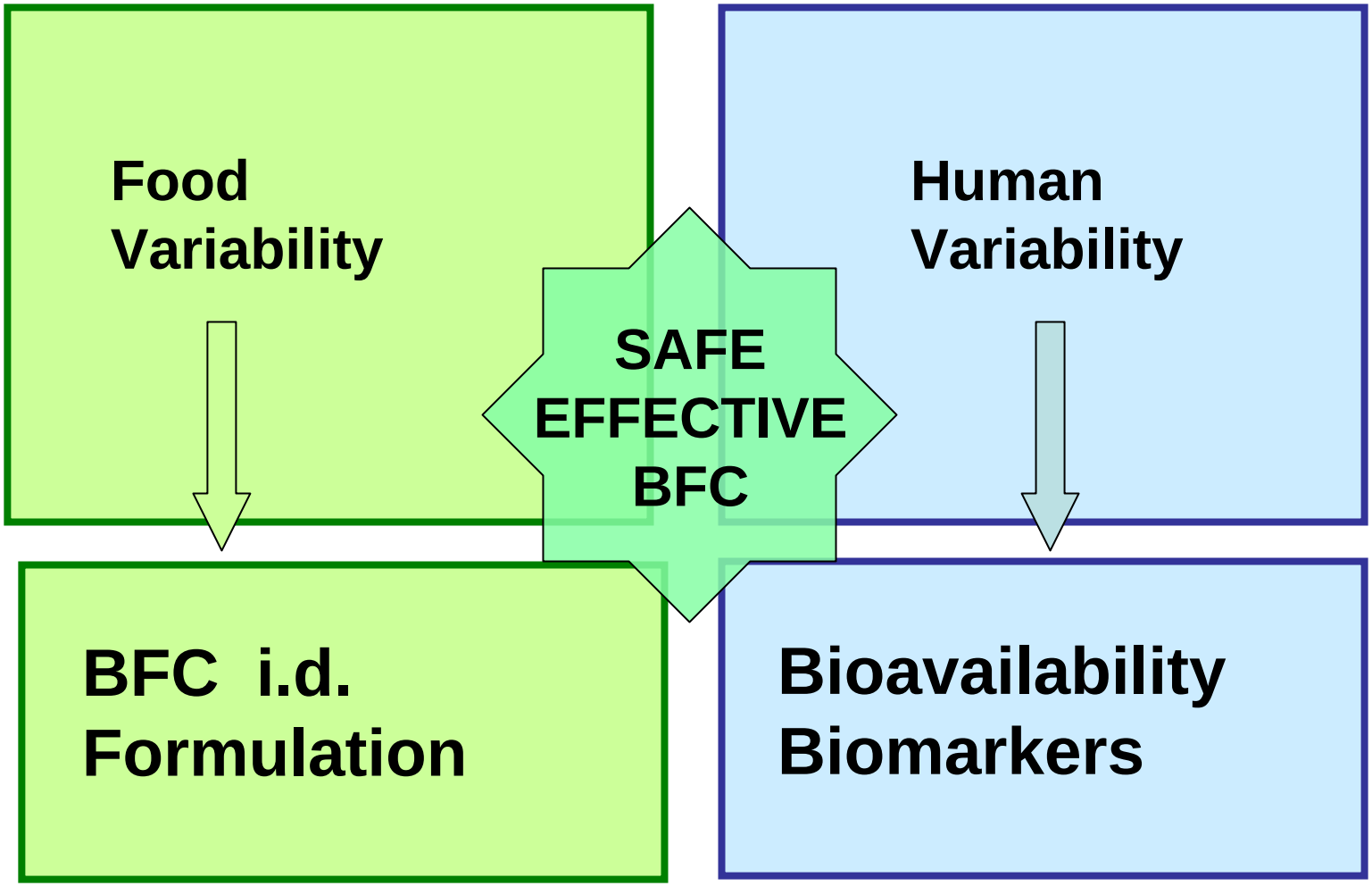

Effective Dose
Plant Genetics
Growing Environment
BFC i.d. Formulation
Bioavailability Biomarkers
Phenotype


Food
Variability
Human
Variability
SAFE
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BFC i.d.
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Food variability

2003 USDA Nutritional Data for RAW BROCCOLI (abridged): Mean value per 100.00 grams edible part

Name	Unit	Amount	%RDA
Food energy	kcal :	28.00	1.0%
Protein	g :	2.98	4.7%
Total lipid (fat)	g :	0.35	0.4%
Carbohydrate	g :	5.240	1.1%
Total saturated fat	g :	0.05	0.2%
Cholesterol	mg :	0.00	0.0%
Total dietary fiber	g :	3.00	12.0%
Vitamin A	IU :	1542.00	15.4%
Ascorbic acid	mg :	93.20	155.3%

Food variability

2005 and 2003 USDA Nutritional Data for RAW BROCCOLI (abridged): Mean value per 100.00 grams edible part

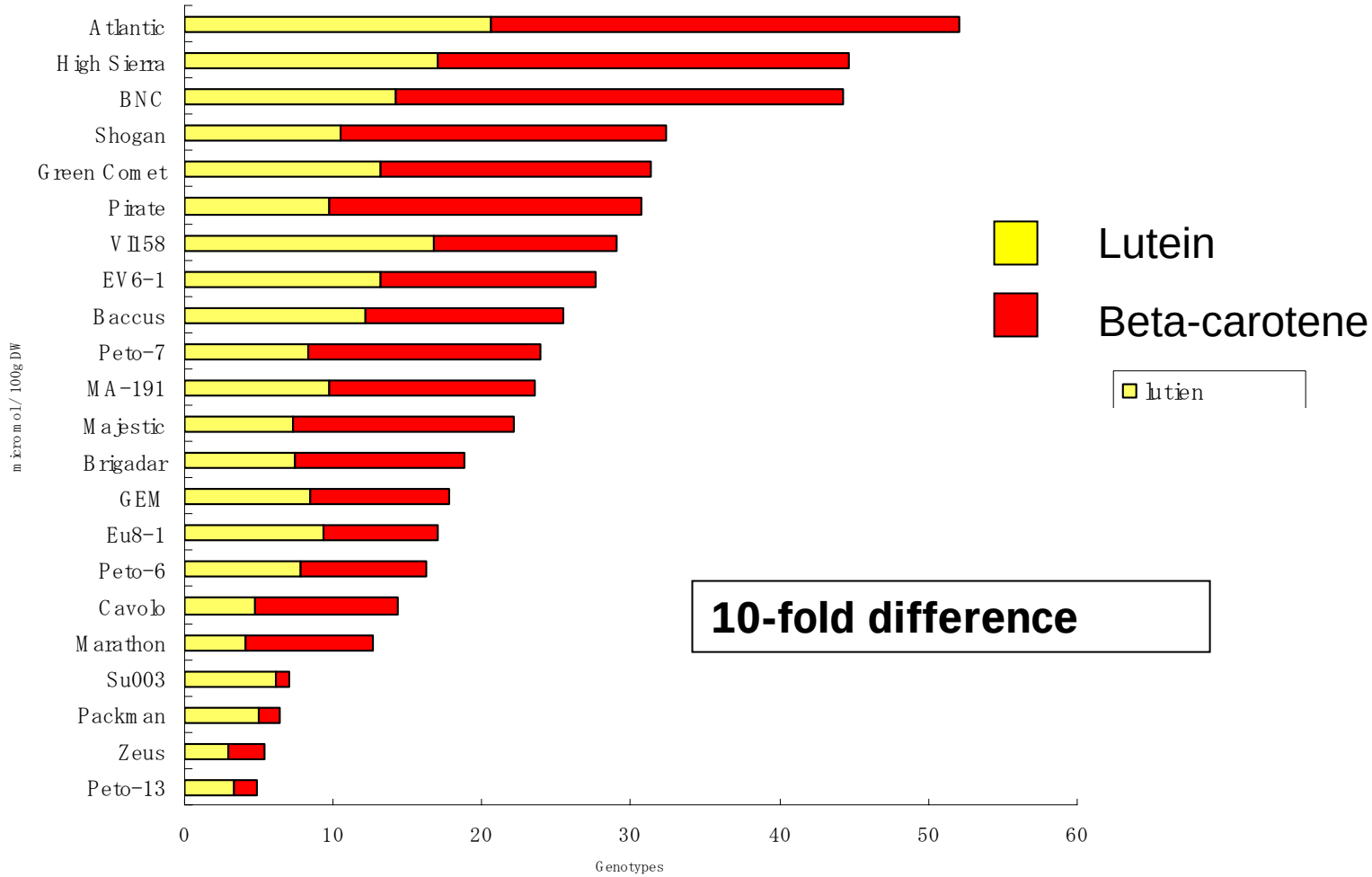
Name	Unit	Amount 2003	Amount 2005	#data points	S.E.
Food energy	kcal:	28.00	28.00	1	
Protein	g :	2.98	2.98	22	.11
Total lipid (fat)	g :	0.35	0.35	22	.03
Carbohydrate	g :	5.24	5.24	1	
Total saturated fat	g :	0.05	0.05	1	
Cholesterol	mg :	0	0	1	
Total dietary fiber	g :	3.0	-		
Vitamin A	IU :	1542	3000	1	
Ascorbic acid	mg :	93.2	93.2	15	2

Mean Vitamin Levels in 50 Broccoli Genotypes
(mg/ 100 g fresh weight)

β-carotene	0.89
range	0.37 - 2.42
α -tocopherol	1.62
range	0.46 - 4.29
Ascorbate	74.7
range	54.0 - 119.8

Kurilich et al, 2001

Carotenoid content of Broccoli

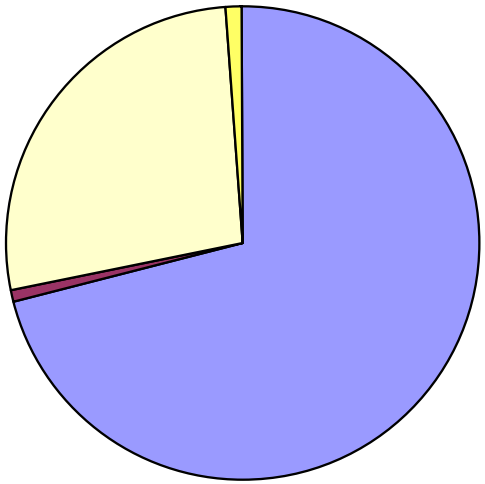


Means, 22 different broccoli genotypes (μmol/100g DW)

Eberhardt et al, in preparation

Variability due to Genotype, Environment or GxE

Lutein

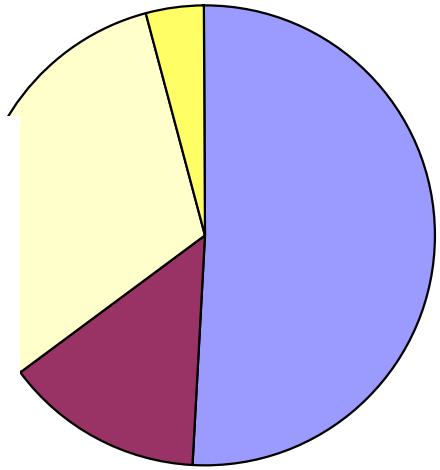


G: 71.2%*

E: <1.0%

GxE: 27.4%*

β-Carotene



G: 50.0%*

E: 13.9%

GxE: 30.6%*

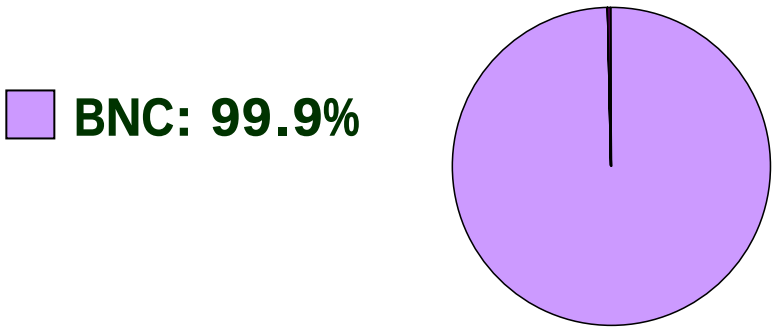
10 broccoli genotypes, 4 environments

* $p<0.05$

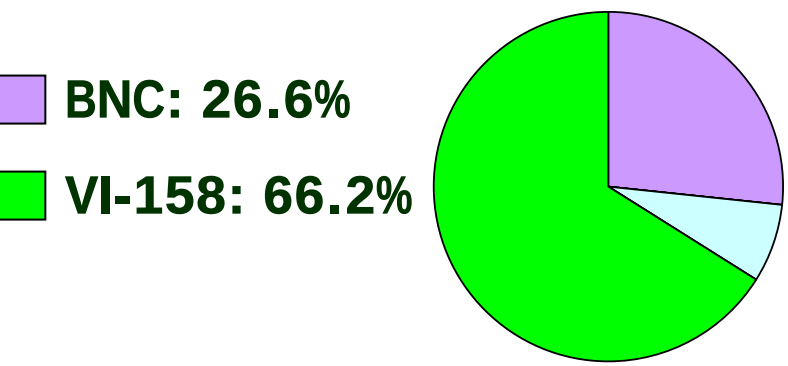
Kobira et al, unpublished

Stability Analysis of GxE variability in 6 genotypes: 2 genotypes accounted for most GxE variability

Lutein



β-Carotene

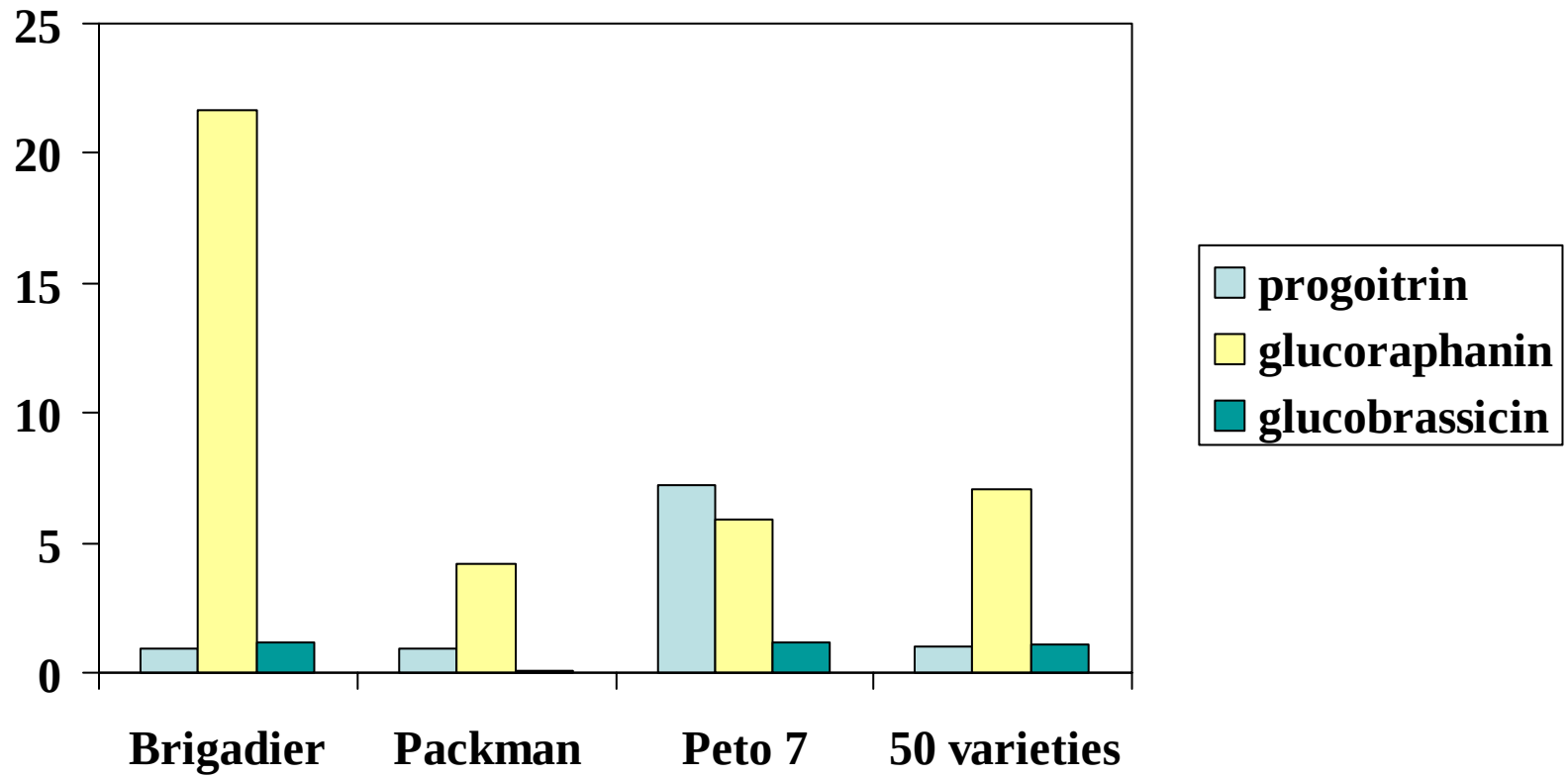


Conclusions:

- **Significant proportion of variance (P<0.05) due to genotype : can breed for higher levels**
- **Significant proportion of GxE variance (P<0.05) due to GxE : need choice of stable varieties as parents in a breeding program**

Kobira et al, unpulished

Glucosinolate variation among 50 broccoli varieties; one season



Kushad et al, 1999

Food variability

3-DAY SPROUTS

single variety on the market, less variation

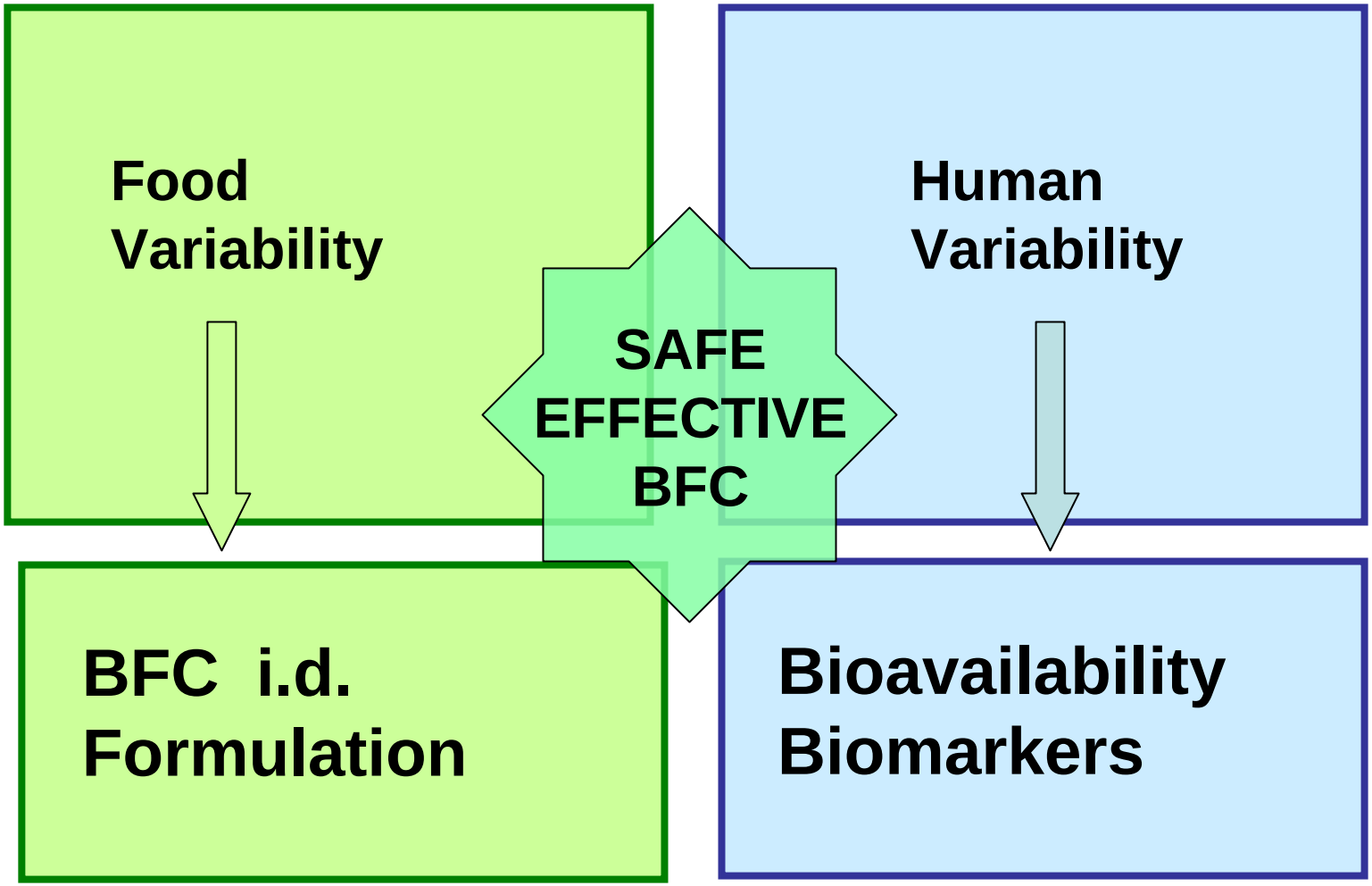
MARKET STAGE BROCCOLI :

Broad variation in content of glucoraphanin

EXTRACT, AS FREEZE-FRIED POWDER or TEA

Least variation: Each lot can be analyzed

For clinical studies, variability can best be overcome by using juices, extracts or freeze-dried preparations.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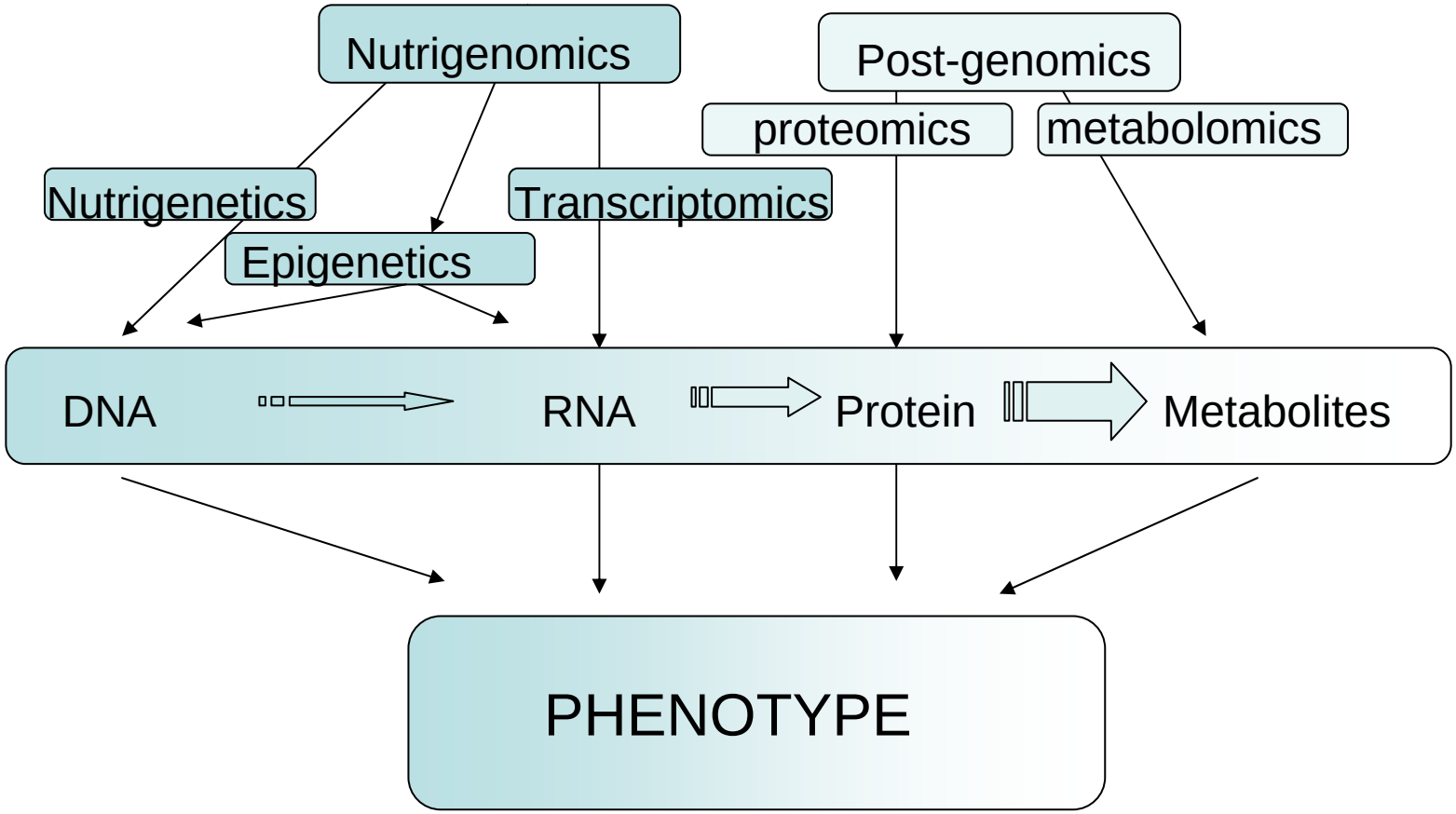
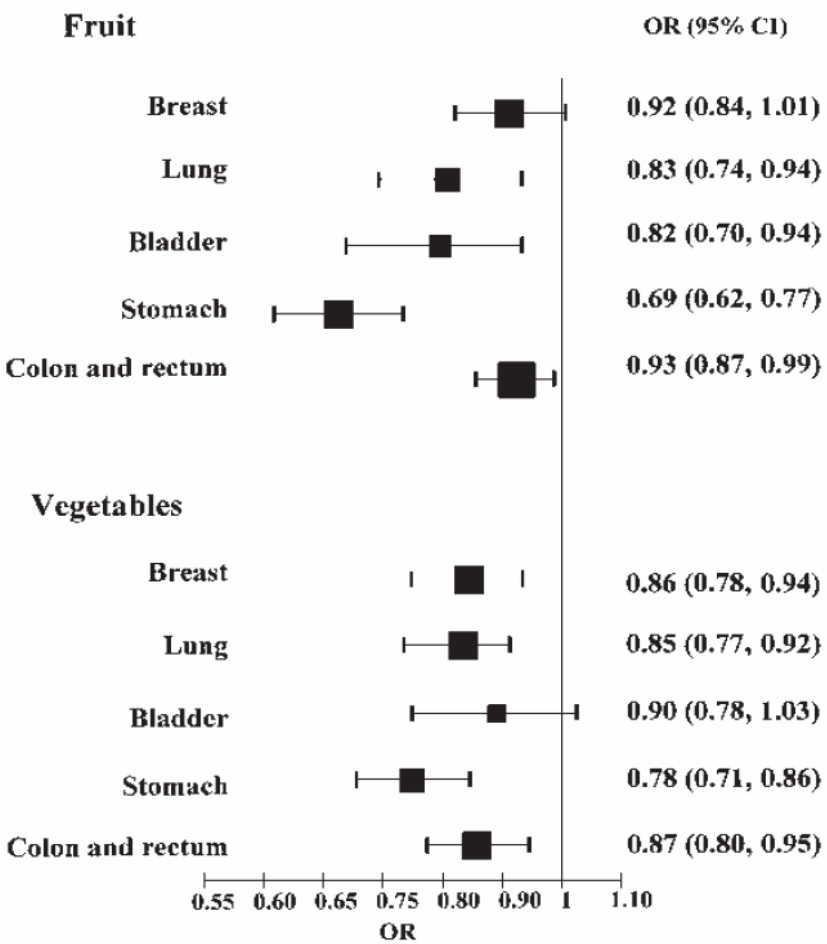
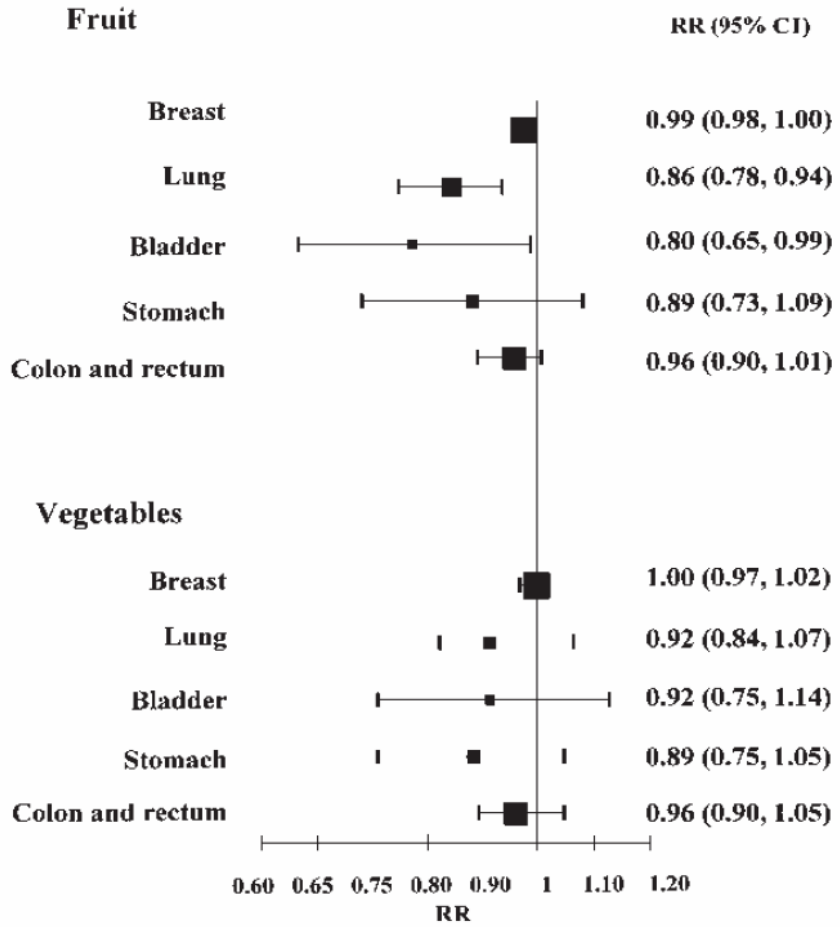

Nutrigenomics
Post-genomics
proteomics
metabolomics
Nutrigenetics
Transcriptomics
Epigenetics
DNA
RNA
Protein
Metabolites
PHENOTYPE

Epidemiology of Dietary Cancer Prevention : Fruit and Vegetables

Case-Control Studies



Cohort Studies



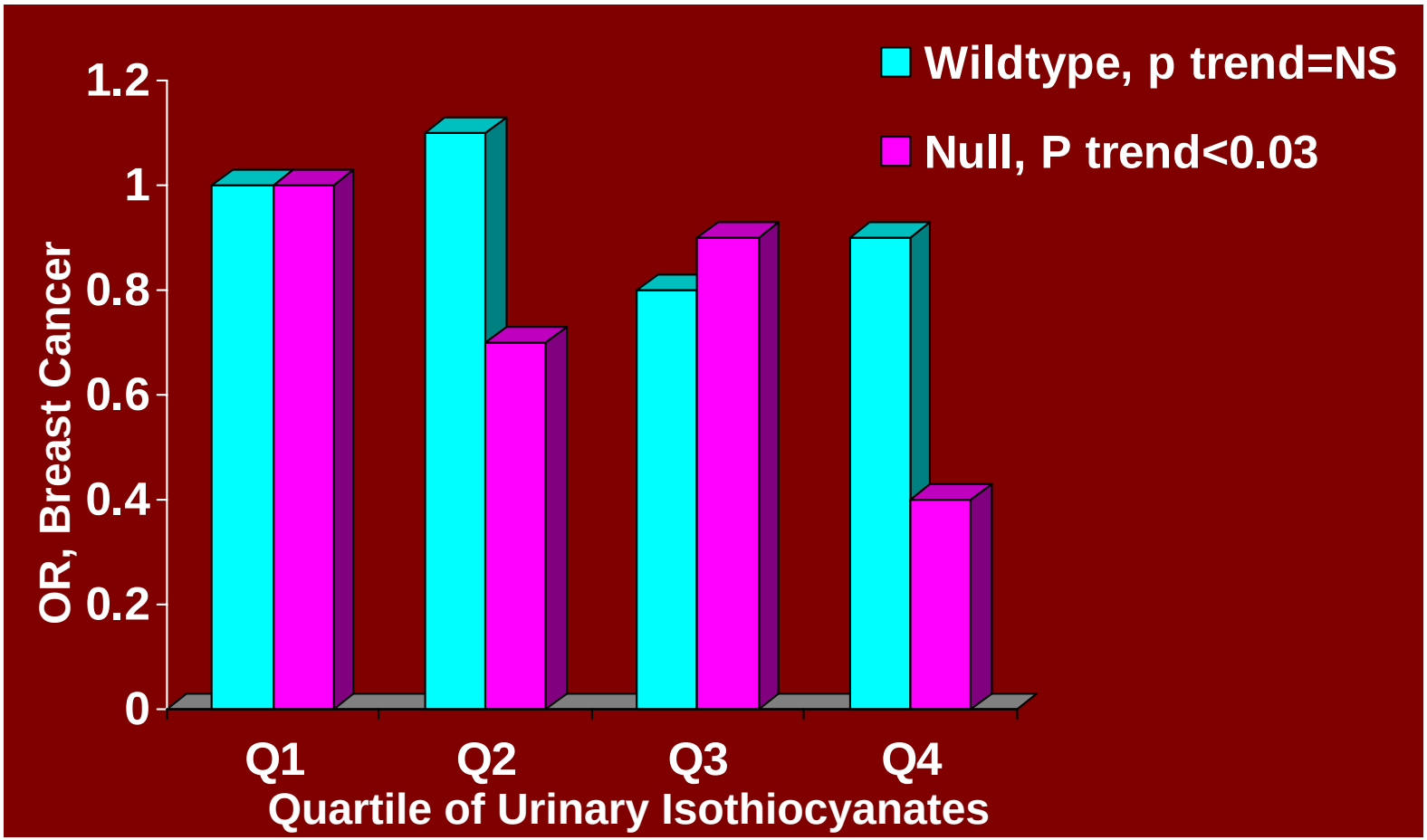
Riboli and Norat, 2003

Crucifers Lower Risk for Lung Cancer More Effectively in those at high risk

	Relative risk
Crucifers / non-smokers	0.70 (ns)
Crucifers / smokers	0.31 ($p<0.05$)

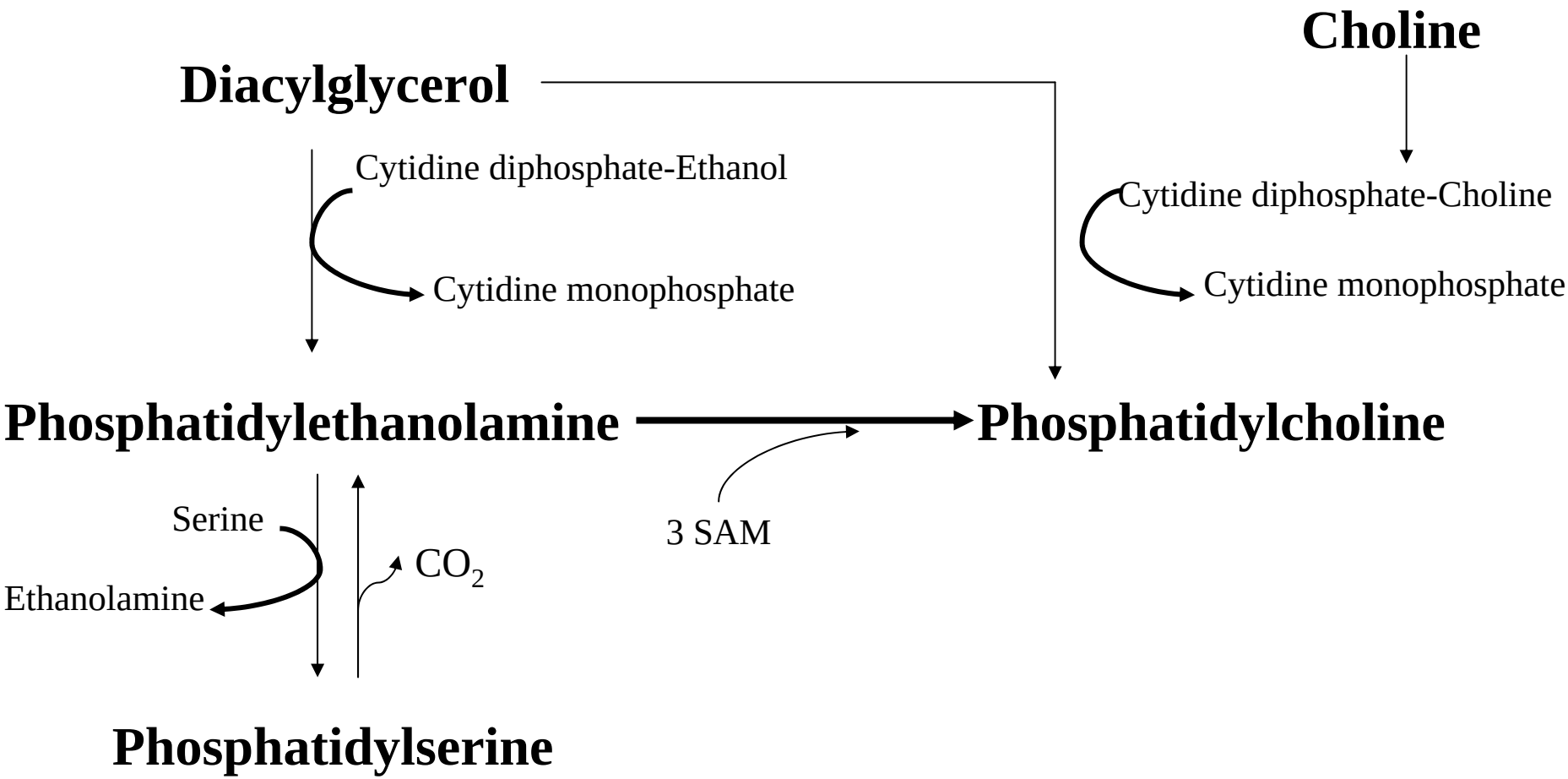
Zhao et al, 2001, Cancer Epi Bio Prev 10:1063-7

Breast Cancer Risk is lowered by dietary Crucifers in those with a GSTT1 Null Phenotype



Fowke et al., Cancer Res. 63: 3980-3986, 2003

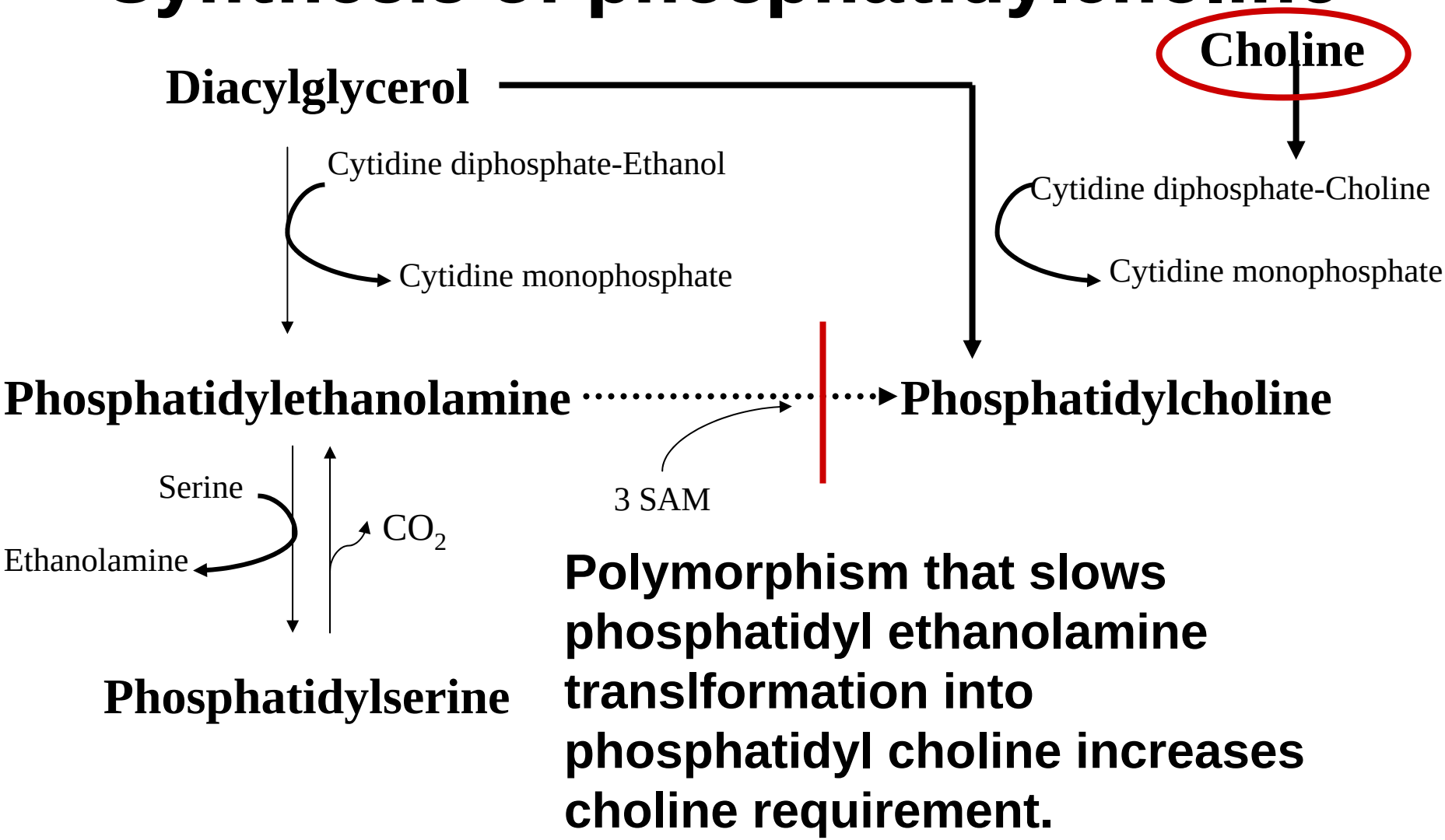
Synthesis of phosphatidylcholine



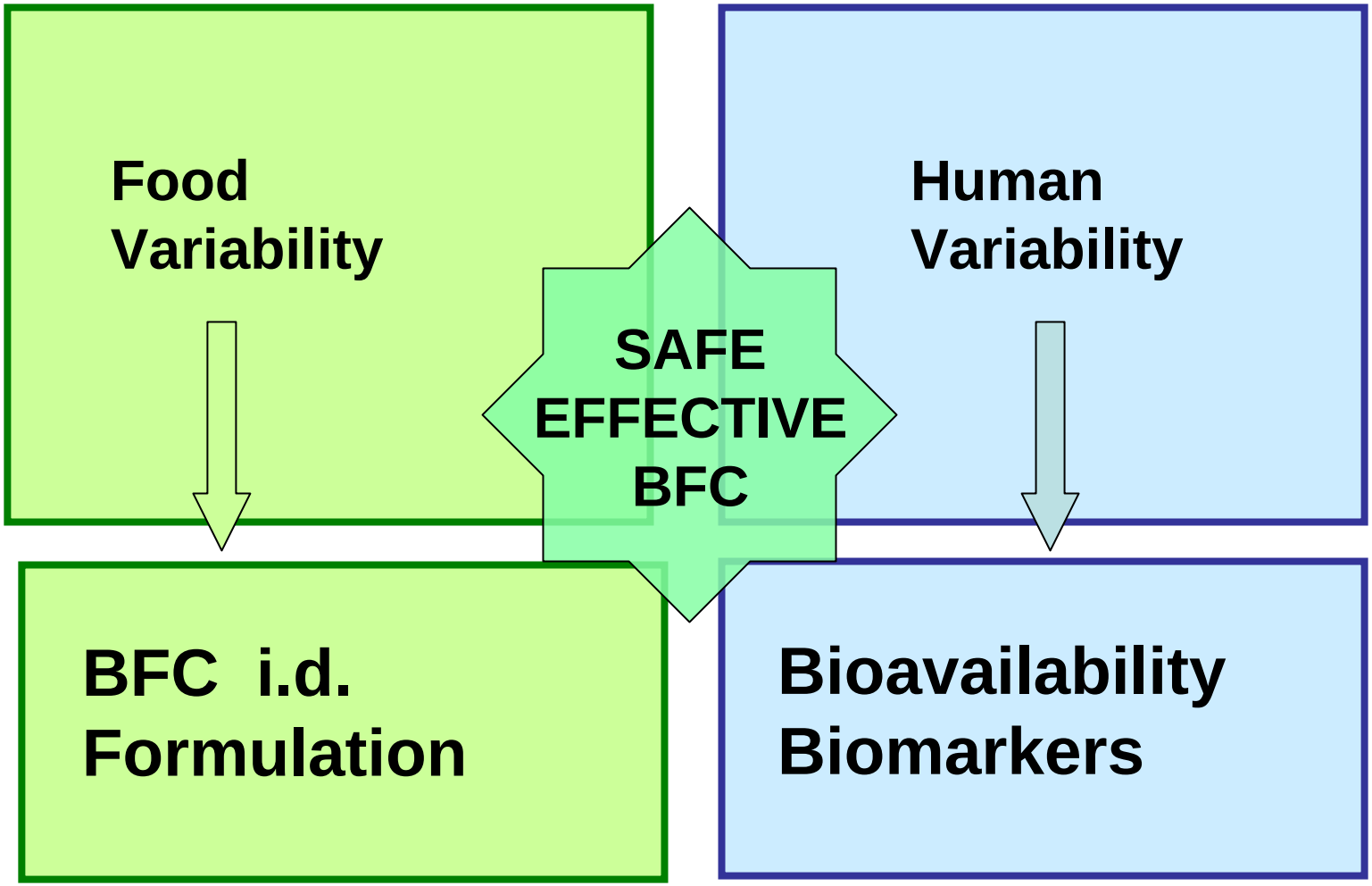
Metabolic redundancy: most pathways have built-in redundancy: here there are two paths for phosphatidyl choline formation

An example of enhanced choline requirement in a few individuals, to meet a metabolic need (choline acting as an essential nutrient) – choline is not acting as a BFC

Synthesis of phosphatidylcholine



Polymorphism that slows phosphatidyl ethanolamine translformation into phosphatidyl choline increases choline requirement.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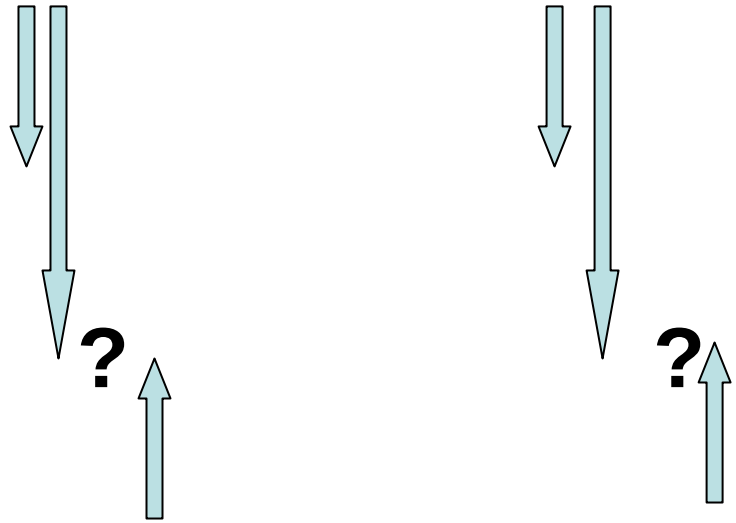
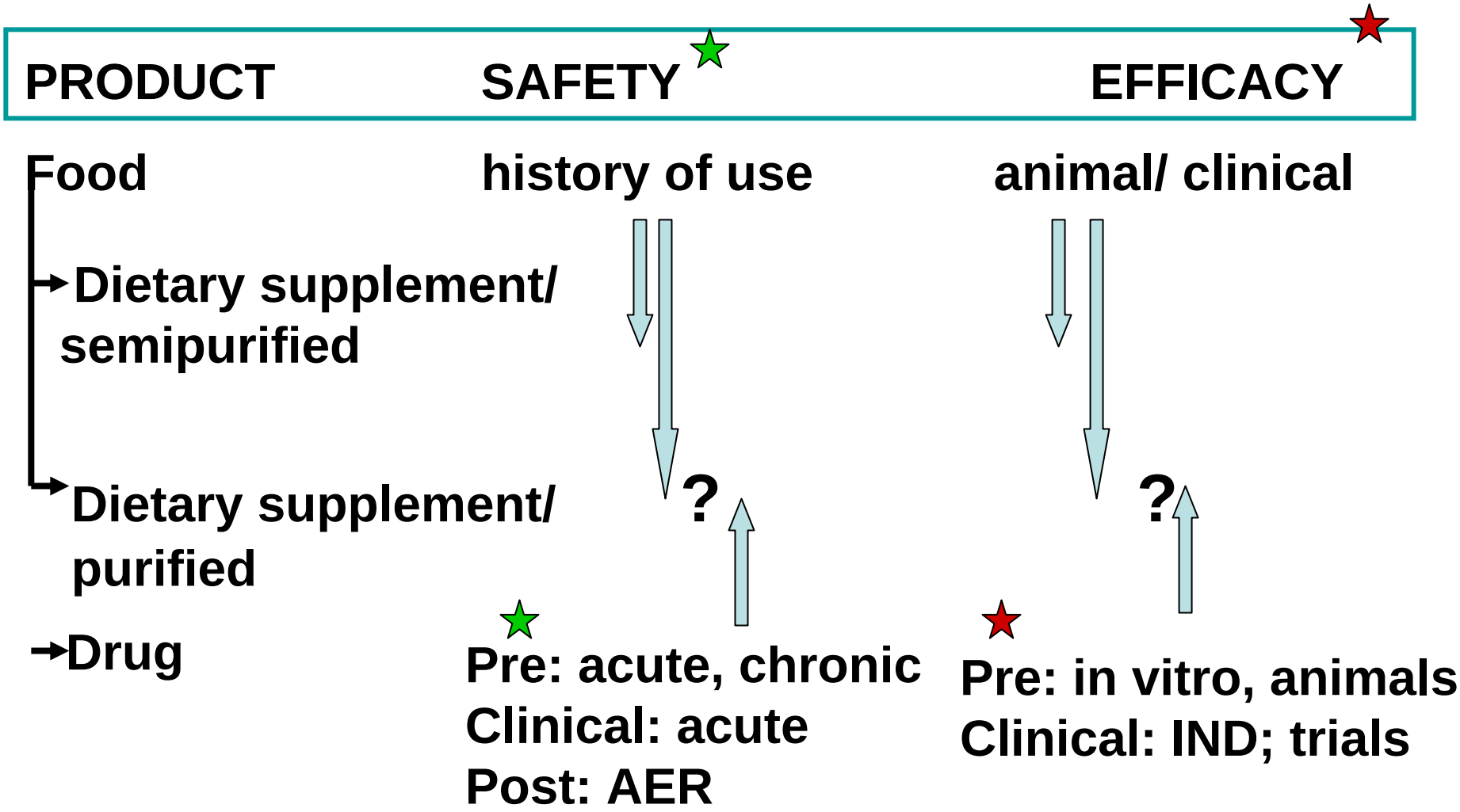
PRODUCT	SAFETY	EFFICACY
Food	history of use	animal/ clinical
Drug	pre/clinical/post	pre/clinical

PRODUCT	SAFETY	EFFICACY
Food	history of use	animal/ clinical
Dietary supplement semipurified BFC	⇓	⇓ Epi & feeding
Dietary supplement purified BFC		

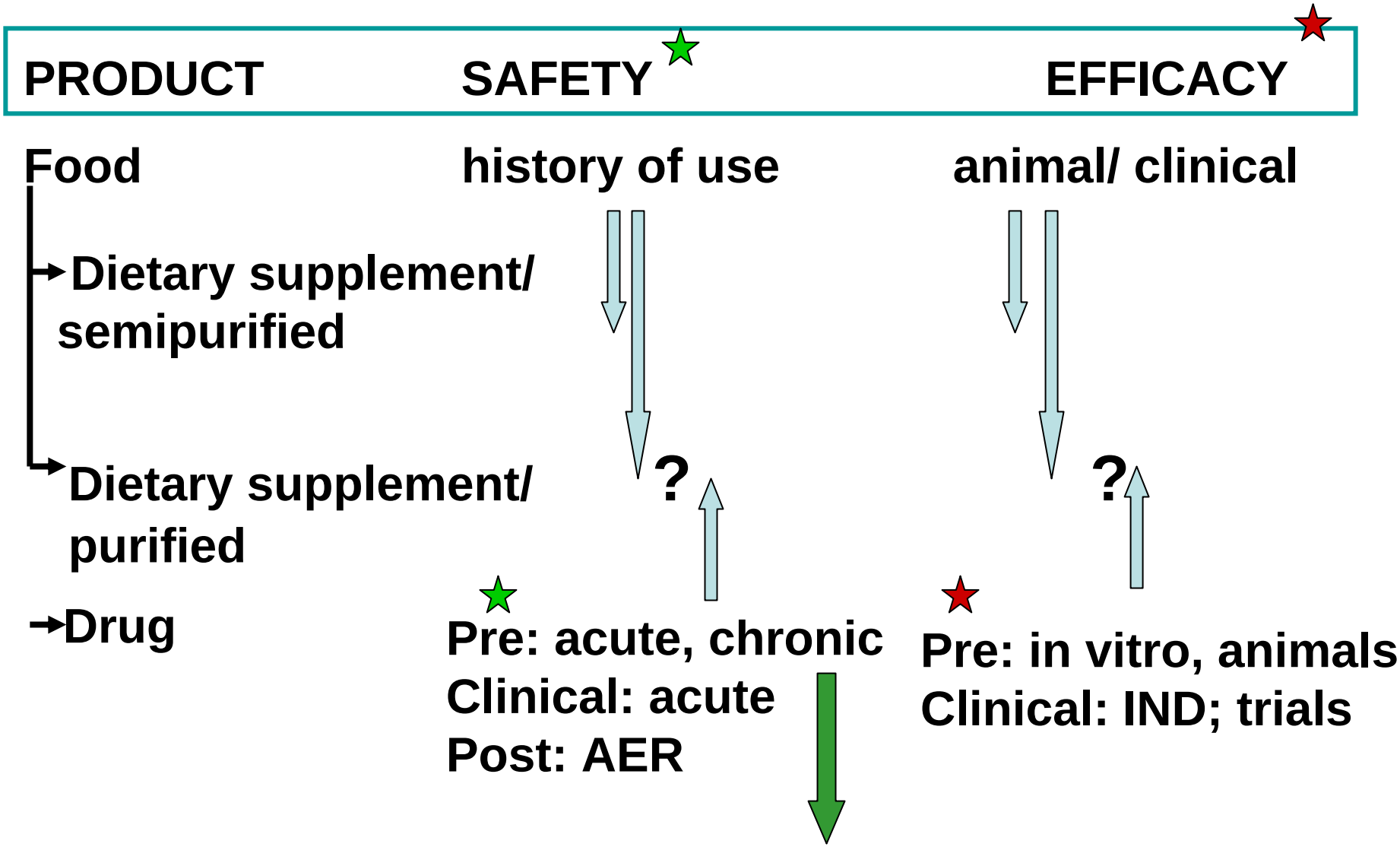
PRODUCT	SAFETY	EFFICACY
Food	history of use	animal/ clinical
→ Dietary supplement/ semipurified		
→ Dietary supplement/ purified	?	?
→ Drug	pre/clinical/post	pre/clinical



If BFC are purified, for supplements or fortification of foods, do we need to provide more safety (or efficacy) evaluation prior to use ?



For drugs, we require acute and chronic testing preclinical, acute clinical evaluation of safety and post-market adverse event reporting


PRODUCT
SAFETY
EFFICACY
Food
history of use
animal/ clinical
Dietary supplement/ semipurified
Dietary supplement/ purified
Drug
?
?
Pre: acute, chronic
Clinical: acute
Post: AER
Pre: in vitro, animals
Clinical: IND; trials
Do we need to develop a safety profile in animal studies, that can act as an early warning system for safety of purified BFC ?